Modulation of T cell responses by IL-2 and IL-2 complexes

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Abstract: Interleukin-2 (IL-2) is a cytokine centrally involved in the regulation of immune tolerance and activation by its effects on CD4+ T regulatory (Treg) cells and cytotoxic effector lymphocytes, respectively. Due to these properties IL-2 immunotherapy has been used, as low-dose IL-2, in the treatment of autoimmune and chronic-inflammatory disorders; conversely, at high doses, IL-2 has shown efficacy in a subset of patients with metastatic cancer. Recent advances have highlighted the possibility of using improved IL-2-based therapies, such IL-2-antibody complexes (IL-2 complexes), able to selectively and potently stimulate either Treg cells or cytotoxic effector cells. This article discusses the properties and clinical implications of IL-2 and IL-2 complexes.

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Modulation of T cell responses by IL-2 and IL-2 complexes

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ABSTRACT

Interleukin-2 (IL-2) is a cytokine centrally involved in the regulation of immune tolerance and activation by its effects on CD4+ T regulatory (Treg) cells and cytotoxic effector lymphocytes, respectively. Due to these properties IL-2 immunotherapy has been used, as low-dose IL-2, in the treatment of autoimmune and chronic-inflammatory disorders; conversely, at high doses, IL-2 has shown efficacy in a subset of patients with metastatic cancer. Recent advances have highlighted the possibility of using improved IL-2-based therapies, such IL-2-antibody complexes (IL-2 complexes), able to selectively and potently stimulate either Treg cells or cytotoxic effector cells. This article discusses the properties and clinical implications of IL-2 and IL-2 complexes.

Background

Lymphocyte homeostasis and function crucially depends on cytokine signals, most notably common γ chain (γ_c) cytokines, which include interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15, and IL-21 (1). Of these cytokines, IL-2 is special in being implicated in immune tolerance and immune activation (2-4). Many immune cells have been reported to produce IL-2, including $CD4^+$ helper T cells, $CD8^+$ T cells, natural killer (NK) cells, NKT cells, activated dendritic cells and mast cells (3). During steady-state conditions, the main source of the low background levels of IL-2 are $CD4^+$ helper T cells, while following immune activation large amounts of IL-2 are synthesised by both $CD4^+$ helper and $CD8^+$ T cells, with some contribution also from the aforementioned other immune cells (3).

IL-2 acts on cells expressing either dimeric IL-2 receptors (IL-2R) consisting of IL-2Rβ (CD122) and the γ_c (CD132) or trimeric IL-2Rs made of CD122 and γ_c plus the IL-2Rα subunit (also called CD25), with trimeric IL-2Rs displaying a 10–100-fold higher affinity for IL-2 compared to dimeric IL-2Rs (5, 6). Dimeric IL-2Rs are most prominent on antigen-experienced (memory) $CD8^+$ T cells and NK cells, while trimeric IL-2Rs are typically expressed at high levels by thymus-derived ('natural') $CD4^+$ T regulatory (Treg) cells and recently-activated effector T cells and, at low to very low levels, on other immune (including group 2 innate lymphoid cells) and non-immune cells (such as endothelial cells, see next chapters) (3, 7, 8). Thus, low doses of IL-2, such as steady-state homeostatic levels, necessitate the expression of high trimeric IL-2R expression levels and appear to mainly support the maintenance of Treg cells (9, 10), which play a key role in peripheral immune tolerance by restricting responses of (autoreactive) effector $CD4^+$ and $CD8^+$ T cells. Conversely, high doses of IL-2 are able to also activate dimeric IL-2Rs and by that virtue strongly stimulate cytotoxic lymphocytes, including $CD8^+$ T and NK cells, in addition to activating Treg cells (11). These observations highlight the somewhat opposed roles of IL-2 in activating and containing immune responses, thereby regulating the ratio of effector T cells to Treg cells (Teff:Treg ratio). In immune pathologies, the lack of therapeutics able to selectively boost Treg cells has – at least until recently – focused clinical management of patients with inflammatory and autoimmune diseases on the use of immunosuppressive agents targeting effector T cells, thus somewhat correcting the Teff:Treg ratio. However, this approach comes with the disadvantage of suppressing both harmful and protective immune responses. Likewise, the use of high-dose IL-2 to stimulate effector T cells against metastatic cancer has shown some success but has also suffered from shortcomings, including the concomitant stimulation of Treg cells able to dampen anti-tumour immune responses as well as the appearance of IL-2-induced toxic adverse events.

For several years, we have been studying modified IL-2 formulations for selective stimulation of either Treg cells for the treatment of autoimmune disorders and graft-related immunopathology or preferential activation of cytotoxic lymphocytes for cancer immunotherapy. We have shown that so-called IL-2-antibody complexes (briefly, IL-2 complexes), consisting of IL-2 conjugated to a particular anti-IL-2 monoclonal antibody (mAb), are able to selectively stimulate either $CD4^+$ Treg cells or $CD8^+$ T and NK cells, depending on the anti-IL-2 mAb used (3, 12-16), thus leading to specific immune modulation without immunosuppression.

$CD4^+$ Treg cell-stimulating IL-2 complexes

As mentioned before, $CD4^+$ Treg cells constitutively express high levels of CD25 (7), which explains the high sensitivity of Treg cells to IL-2 *in vitro* and *in vivo* (9, 17-22). Thus, Treg cell homeostasis depends on IL-2 signals *in vivo* and animals and humans deficient in IL-2, CD25 or CD122 develop systemic autoimmune disease owing to a reduction in Treg cell numbers (2-4, 10, 22), highlighting the central role of IL-2 in Treg cell development, survival and function. IL-2 is also important in Treg cells for the upregulation of CD25 and the maintenance of the signature transcription factor forkhead box P3 (FoxP3), both for thymus-derived ('natural') and peripherally-derived ('induced') Treg cells (2, 3, 23-26).

As for humans, low-dose IL-2 immunotherapy was recently tested in patients with chronic graft-*versus*-host disease (GvHD) as well as in patients with hepatitis C virus-induced cryoglobulinaemic vasculitis. These disorders are associated with low Treg cell numbers as assessed by circulating Treg cell counts, thus resulting in an imbalance of the Teff:Treg ratio, which has been proposed to underly the immunopathology observed with these disorders that are usually treated using immunosuppressive agents. The new studies showed that low-dose IL-2 resulted in an increase in peripheral blood Treg cell counts, which in chronic GvHD was associated with disease improvement in 12 out of 23 and disease stabilisation in the remainder of the treated patients (27, 28), and in cryoglobulinaemic vasculitis resulted in improvement in 8 out of 10 treated patients (29), whereas no apparent stimulation of effector T cells or severe side effects occurred (10).

With the use of Treg cell-selective IL-2 complexes, such as IL-2/JES6-1 complexes (12) or IL-2/5344 complexes (14), IL-2 can be targeted in a very directed manner to $CD25^+$ $FoxP3^+$ Treg cells. This selectivity stems from the covering of the CD122/γc-binding site of IL-2 by the anti-IL-2 mAb, thus allowing only cells expressing very high levels of CD25, *i.e*. Treg cells under steady-state conditions, to recognise and compete for these IL-2 complexes, as cells expressing only dimeric IL-2Rs cannot utilise their CD122 anymore to bind IL-2 in this form (3). Use of Treg cell-expanding IL-2 complexes proved to be beneficial for the treatment of several mouse models of pathologic inflammation or autoimmunity, including a T cell-mediated form of asthma (30), autoimmune diabetes in non-obese diabetic mice (31), experimental autoimmune encephalomyelitis (13), experimental myasthenia (32), and collagen-induced arthritis (33). Moreover, use of a short course of Treg cell-specific IL-2 complexes prior to transplantation induced long-term acceptance of fully major histocompatibility complex (MHC)-mismatched pancreatic islet grafts in over 80% of recipient mice (13). Based on these data, generation and development of appropriate JES6-1-type anti-human IL-2 mAbs and IL-2 complexes could prove valuable for the treatment of chronic inflammatory and autoimmune disorders (3, 10, 34).

$CD8^+$ and NK cell-stimulating IL-2/mAb complexes

In contrast to low-dose IL-2, high-dose IL-2 immunotherapy has been used already since the early 1980s in the treatment of cancer, leading to clinical responses in 13–16% of patients with metastatic melanoma and metastatic renal cell carcinoma (35, 36), with as much as 5–10% of these patients surviving for 20 years and more. Based on these data, high-dose IL-2 treatment was recommended as a first-line therapy in certain stage IV melanoma patients (37). The rate of overall survival could be improved by using combined vaccination strategies or higher doses of IL-2, the latter of which is hampered by the development of dose-dependent IL-2-induced side effects, termed vascular leak syndrome (VLS), which resembles the clinical manifestations of septic shock (34, 38, 39). Another potential disadvantage of IL-2-based cancer immunotherapy relates to the property of IL-2 in promoting homeostasis and survival of Treg cells, which are able to inhibit or dampen T cell responses to self-antigens, including tumour antigens (20, 40).

To counter these disadvantages, modified IL-2 formulations have been generated, including mutated IL-2 variants, IL-2 fusion proteins and $CD8^+$ T and NK cell-directed IL-2 complexes (41). We favour IL-2 complexes, as the binding of the mAb protects IL-2 from scavenger molecules and from interacting with and damaging endothelial cells (3, 15); once IL-2 complexes have reached their target cells (*i.e*. cytotoxic $CD8^+$ T cells and NK cells), unmodified IL-2 can interact with and fully stimulate the IL-2R and thus the effector cells (3, 15).

In our original work, we demonstrated that the combination of recombinant mouse IL-2 with a particular anti-mouse IL-2 mAb (clones S4B6 or JES6-5H4) led to the formation of IL-2 complexes, which markedly enhanced the *in vivo* activity of IL-2, thus leading to vigorous expansion of $CD8^+$ T cells and NK cells, while $CD4^+$ $CD25^+$ $FoxP3^+$ Treg cells were barely stimulated by this form of IL-2 (12). This principle also applied to other cytokines, including IL-4, IL-6, IL-7 and IL-15 (42-46). Subsequently, cytokine complexes using IL-2, IL-4, IL-7 and IL-15 were assessed in different murine tumour models where IL-2 complexes appeared to show the most favourable anti-tumour properties (15, 16, 41, 47). Most notably, IL-2 in the form of IL-2 complexes was not only more potent in stimulating $CD8^+$ T cells and NK cells but also caused significantly less adverse effects compared to standard high-dose IL-2 (15). Accordingly, administration of IL-2 complexes

to normal mice led to marked expansion of endogenous $CD8^+$ T and NK cells, which exhibited potent anti-tumour responses against syngeneic B16F10 melanomas, growing either intradermally or as pulmonary nodules following intravenous injection, as well as intradermal nodules of MC38 colon carcinoma or LLC Lewis lung carcinoma (15, 16). Use of IL-2 complexes at a low concentration of IL-2, *i.e.* 5,000 international units (IU) per injection, generated an anti-B16F10 response that was comparable to the therapeutic effect achieved by using the highest tolerated dose of conventional IL-2 immunotherapy, *i.e.* 200'000 IU per injection. However, in contrast to 200,000 IU IL-2, which caused significant toxicity (*i.e.* VLS), such as prominent pulmonary oedema and significant liver cell damage, IL-2 complexes at 5,000 IU caused only negligible side effects.

These improved therapeutic features of IL-2 complexes stimulating $CD8^+$ T and NK cells are due to reversible modifications of IL-2 following anti-IL-2 mAb binding, thus leading to the selective direction of IL-2 towards cells expressing high levels of the intermediate-affinity IL-2R consisting of CD122 and γ_c, but do not depend on CD25 expression (3, 12, 14, 15, 34). The underlying mechanism appears to rely on the covering of the CD25-binding epitope of IL-2 by this type of anti-IL-2 mAb (3, 12, 14, 15, 34). Therefore, cells expressing high levels of CD25 and depending on CD25 for their IL-2 binding, such as $CD4^+$ $CD25^+$ Treg cells and endothelial cells, do not become efficiently stimulated by such IL-2 complexes, thereby disfavouring the establishment of an immunosuppressive environment and circumventing the generation of endothelial cell damage leading to VLS (3, 15, 22). Additionally, IL-2 complexes show a roughly 20-40 times increased *in vivo* half-life compared to IL-2 (3, 14, 41), thereby providing a more even and sustained stimulation of IL-$2R^+$ immune cells.

Collectively, based on preclinical data IL-2 complexes show promising effects in several models of immunopathology and cancer, thus warranting development of appropriate clinical-grade IL-2 complexes.

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